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(54) **WILD APPLE POLYPHENOL AND PROCESS FOR PRODUCING THE SAME**

(57) A fruit polyphenol obtained by subjecting a crab apple fruit to pressing and/or extraction. The fruit polyphenol is rich in such components as condensed tannins (procyanidins), chlorogenic acid, and epicatechin. The fruit polyphenol can be produced economically and efficiently.

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## Description

## TECHNICAL FIELD

**[0001]** The present invention relates to a fruit polyphenol derived from a crab apple fruit, out of polyphenols known to have a variety of functionalities, and a production process thereof.

## BACKGROUND ART

**[0002]** A wide variety of polyphenols known as secondary metabolites of plants are present in the plant kingdom. Some of these polyphenols have been found to show diversified physiological activities. It has heretofore been disclosed that catechin contained in tea has physiological activities such as antibacterial activity, antioxidative activity, anticancerous activity, antiallergic activity, deodorizing activity, antiviral activity and blood cholesterol oxidation inhibiting activity (See JP-A-63-214183, JP-A-2-6499, JP-A-4-178320, etc.).

**[0003]** Further, in JP-A-7-285876, it is reported that polyphenols contained in extracts of unripe apples, unripe pears or unripe peaches have activities such as antioxidative activity, blood pressure reducing activity, antimutagenic activity, antiallergic activity, anticariogenic activity and deodorizing activity. In addition, in "Japanese and Chinese Medicine - New Edition" (author: Kaneyoshi Akamatsu, Ishiyaku Syuppan Kabushiki Kaisha, published in 1980, pp. 359 to 360, J. Liquid Chromatography, 15(4), 637 to 646(1992)), it is mentioned that apples contain tannins. However, all the mention is for apples to be eaten fresh or apples to be processed, and no mention is made of crab apples.

**[0004]** Thus, it has been found that tea and unripe fruits, for example, have diversified physiological activities. The present inventor has made intensive studies on a process for producing a polyphenol having a wide variety of physiological activities economically and efficiently and on raw materials. As a result, the inventor has found that crab apples contain a significantly large amount of polyphenols, particularly a condensation tannin (procyanidin). The present invention has been completed based on this finding.

## DISCLOSURE OF THE INVENTION

**[0005]** That is, according to the present invention, a fruit polyphenol is provided which is obtained by subjecting a crab apple fruit to pressing and/or extraction.

**[0006]** Further, according to the present invention, a process for producing a fruit polyphenol is provided which comprises the steps of subjecting a crab apple fruit to pressing and/or extraction and then purifying the resulting juice and/or extract so as to obtain a polyphenol fraction.

**[0007]** In the present invention, it is preferable to add sulfurous acid in a concentration of 350 to 3,000 ppm at the time of pressing a crab apple fruit. Further, when a crab apple fruit is subjected to extraction, it is preferable to add sulfurous acid in a concentration of 100 ppm or higher if a proper amount of alcohol is mixed into the crab apple fruit which is then crushed. Then, after a clear extract is prepared, sulfurous acid is preferably added in a concentration of 50 ppm or higher so as to prevent oxidation.

## BEST MODE FOR CARRYING OUT THE INVENTION

**[0008]** Hereinafter, the present invention will be described in detail.

**[0009]** A fruit polyphenol in the present invention comprises a polyphenol fraction obtained by subjecting a crab apple fruit to pressing and/or extraction and then purifying the obtained juice or extract. The purification to obtain the polyphenol fraction is carried out by treating the juice or extract with an adsorbent, and a fraction adsorbed to the adsorbent (hereinafter referred to as "adsorbed fraction") contains a polyphenol. Then, the adsorbed fraction is eluted with an anhydrous alcohol (such as ethanol) so as to obtain a purified polyphenol fraction.

**[0010]** This polyphenol fraction can be further concentrated to obtain a liquid preparation. Further, when the concentrated polyphenol fraction is subjected to spray-drying or freeze-drying, a powdery preparation can be obtained.

**[0011]** As a raw material of the polyphenol of the present invention, a crab apple fruit is used. An apple is a plant belonging to Malus of Rosaceae, and a crab apple refers to a species which has not been subjected to breeding by a human or a species which is not or cannot be eaten fresh or processed. Further, as the raw material of the polyphenol of the present invention, a ripe fruit and an unripe fruit can be used.

**[0012]** Illustrative examples of species of crab apples include Hu Bei Hai Tang, Yin Gui Hai Tang, Xi Fu Hai Tang, Zhong Guo Ping Guo, Cui Guo, Ya Hai Tang, Ya Sha Guo and Ya Ping Guo which are native to China, Adams Crab, Geneva, Gorgeous, Jay Darling, Eleyi, Eley Purple Crab, Malus Robusta, Makamic Crab, Profusion, Royalty, Liset Crab, Red Splendor Crab, Tar Tan, and Lemoine Purple Crab.

**[0013]** It is said that apples are originated in the Caucasus Province near the Caspian Sea. It is known that a vast

number of crab apples have grown in areas from the Uyghur Province in China to Kazakhstan and Uzbekistan bordering the Caspian Sea since a long time ago. Particularly, in the vicinity of the Tian Shan which extends from Shin-gen and I-rei in the Uyghur Province in China to the eastern part of Kazakhstan, a large-scale old growth forest of crab apples spreads. Crab apples in this area have hardly been used by humans. The crab apple contains a large amount of preferable polyphenol component and can therefore be suitably used in the present invention.

[0014] Further, also in Europe and North America, trees of crab apples which are apples without breeding are still observed in many places. These crab trees can also be suitably used in the present invention.

[0015] As a pressing method, there can be used a method which comprises cleaning an apple fruit as a raw material, crushing and pressing the apple fruit with or without addition of sulfurous acid so as to obtain a juice, preferably adding a pectolytic enzyme, and then subjecting the resulting mixture to means such as centrifugation or filtration so as to obtain a clear juice. Meanwhile, as an extraction method, there can be used a method which comprises adding sulfurous acid to a cleaned raw material as required, mixing the material with alcohol (such as ethanol or methanol), crushing the mixture, extracting the resulting material while the material is being immersed and pressed or being refluxed under heating, concentrating the extract under a reduced pressure so as to remove the alcohol, and subjecting the concentrate to centrifugation and filtration or to distribution with an organic solvent such as hexane or chloroform and filtration so as to obtain a clear extract.

[0016] As described above, in the present invention, sulfurous acid is preferably added at the time of pressing and/or extraction. In general, when an apple is pressed to obtain a juice as described above, an appropriate antioxidant is added so as to prevent the juice from being discolored brown by oxidation. Illustrative examples of conventionally used antioxidants include sulfurous acid and vitamin C. A commonly used concentration of sulfurous acid is 200 ppm or lower. Further, to attain an antioxidative effect that is nearly the same as that of sulfurous acid, vitamin C must be added in a concentration of 1,000 ppm or higher. This is because the concentration of sulfurous acid used in production of alcoholic beverages (such as fruit wine) is lower than 350 ppm which is a maximum amount permitted to be added by the liquor tax law and it is generally not attempted to add sulfurous acid in a concentration of 350 ppm or higher. When sulfurous acid is added in a concentration of 350 ppm or higher, the obtained juice has a strong odor of sulfurous acid and cannot be drunk.

[0017] However, as a result of intensive studies made by the present inventor, it has been found that when sulfurous acid is added in a concentration of 350 to 3,000 ppm, more preferably 1,000 to 2,000 ppm, more specifically, when a sulfite such as potassium metabisulfite is added in a concentration of 350 to 3,000 ppm, more preferably 1,000 to 2,000 ppm in terms of sulfurous acid at the time of pressing an apple so as to obtain a juice, a polyphenol component which is intrinsically contained in the apple can be extracted without damaging the component. When the concentration of sulfurous acid is lower than the above range, the obtained juice undergoes an oxidation reaction, and the contents of low-molecular-weight components [such as catechin, epicatechin, procyanidin B1 (PB1), procyanidin B2 (PB2) and procyanidin C1 (PC1)] are significantly lowered although the total content of all polyphenols in the obtained juice does not change. Although details of the reaction are unknown, it is assumed that small molecules are polymerized and modified, because the total polyphenol content hardly changes.

[0018] As for vitamin C, when it is added in a concentration of 3,500 to 30,000 ppm, more preferably 10,000 to 20,000 ppm, it can produce the same effect as that obtained by addition of sulfurous acid in the above concentration range.

[0019] Meanwhile, when an extraction method is employed, sulfurous acid is preferably added in a concentration of 100 ppm or higher, when a proper amount of alcohol is mixed into a crab apple fruit which is then crushed. Then, as described above, after the alcohol is removed and centrifugation and filtration or distribution and filtration are carried out to obtain a clear extract, sulfurous acid is preferably added in a concentration of 50 ppm or higher so as to prevent oxidation.

[0020] As for a purification method, the above clear juice or clear extract is passed through a column filled with an adsorbent capable of selectively adsorbing a polyphenol and releasing the adsorbed phenol by use of an eluant, such as a styrene-divinylbenzene type synthetic adsorption resin, an anion exchange resin or an octadecyl group chemically bonded silica gel (ODS) so as to adsorb a polyphenol fraction. Then, water is passed through the column for washing. Then, a 10-100% alcohol (e.g. ethanol) solution, preferably an about 50% alcohol solution is passed through the column, whereby the polyphenol fraction can be eluted and recovered. The obtained polyphenol solution is concentrated under a reduced pressure so as to remove the alcohol, whereby a fruit polyphenol liquid preparation can be obtained. The liquid preparation may contain an organic acid such as malic acid, a fatty acid ester or the like. Further, the liquid preparation may be spray-drying or freeze-drying either as it is or after an auxiliary agent for powdering such as dextrin or the like is added, whereby a fruit polyphenol powdery preparation can be obtained.

[0021] The fruit polyphenol obtained in the present invention comprises caffeic acid derivatives, p-coumaric acid derivatives, flavan-3-ols (catechins), flavonols (quercetin glycosides), dihydrochalcones (phloretin glycosides) and the like as simple polyphenol compounds and condensed tannins (procyanidins) and the like as high-molecular polyphenol compounds. A polyphenol obtained from a crab apple contains a particularly large quantity of condensed tannins.

[0022] Further, crab apples of some type contain a specific polyphenol component in particularly large quantity, and

conversely, crab apples of some other type contain almost no specific polyphenol component. By use of these crab apples, purification of the specific polyphenol component can be carried out very efficiently.

[0023] The fruit polyphenol obtained in the present invention has a variety of physiological activities. The functionalities of a polyphenol derived from an apple are disclosed in JP-A-7-285876. The polyphenol in the present invention has the activities described in the publication, i.e., antioxidative activity, blood pressure reducing activity, antimutagenic activity, antiallergic activity, anticariogenic activity and deodorizing activity.

[0024] Therefore, the fruit polyphenol produced as described above can be used in drugs as agents having antioxidative activity, blood pressure reducing activity, antimutagenic activity, antiallergic activity, anticariogenic activity and deodorizing activity. Drugs containing the fruit polyphenol can be prepared by a known method in the form of oral drugs such as a tablet, powders, granules, a capsule and a syrup or parenteral drugs such as a suppository, ointment, nebula and injection.

[0025] Further, the fruit polyphenol can be added to beverages and foods in general so that they can be suitably used as beverages and foods having antioxidative activity, blood pressure reducing activity, antimutagenic activity, antiallergic activity, anticariogenic activity and deodorizing activity. To be more specific, the fruit polyphenol can be added to alcohol beverages, carbonated beverages, fruit beverages, lactic acid bacteria beverages, coffee, tea, ice cream, candies, chewing gums, snacks, breads, noodle, and the like.

[0026] Furthermore, the fruit polyphenol can also be added to cosmetics. Illustrative examples of cosmetics to which the fruit polyphenol can be added include skin-care cosmetics and bath cosmetics such as soap, facial wash, cream, skin milk, skin lotions, powders, perfume and lipsticks; hair-care cosmetics such as shampoo and rinse; and toothpaste.

[0027] Most of the various functionalities of the above fruit polyphenol according to the present invention are ascribable to procyanidin fractions. As described in the above description of the production process, these fractions may be oxidized and modified during production. Thus, it is important to carry out a purification step in a short time with the oxidation suppressed as much as possible. When a crab apple containing a larger amount of procyanidin than an unripe apple fruit or a crab apple having composition leaning to a specific component is used, the production step can be simplified, and separation and purification can be carried out in a shorter time.

[0028] Further, in the case of unripe fruits of cultivated species such as "Fuji", apple fruits cultivated with special care must be collected by thinning one by one carefully during their growth. Meanwhile, in the case of crab apples, they grow naturally in the field, neither a fertilizer nor a pesticide must be used for cultivation thereof, and neither efforts nor costs are required. Further, since no pesticide is used, the crab apples are high in the safety. In addition, upon harvesting of the crab apples, unlike thinning works of unripe fruits of cultivated species, all fruits on a tree can be collected. Consequently, the harvesting work is quite easy.

[0029] Hereinafter, the present invention will be further described with reference to Examples. The present invention, however, shall not be limited by these Examples in any way.

#### [Example 1]

##### [General Compositional Analysis of Crab Apple Juice]

[0030] Ripe crab apples were subjected to general compositional analysis.

[0031] Samples: Various ripe crab apples shown in Table 1 and a ripe apple "Fuji" as a comparison were used.

[0032] Production Process of Samples: The fruit samples were crushed in a mixer and pressed while 1,000 ppm in terms of sulfurous acid of potassium metabisulfite was being added thereto as an antioxidant. The obtained juices were subjected to centrifugation and then filtration so as to obtain clear juices. The obtained clear juices were measured for the following items, and the results of the measurements are shown in Table 1.

##### (Measurement Items)

##### [0033]

- Average Weight of Individual Fruit
- Acidity (in terms of malic acid)
- Brix
- Concentration of Saccharose : Measured by high-performance liquid chromatography
- Concentration of Glucose : Measured by high-performance liquid chromatography

Table 1

Kind	Average Weight of Individual Fruit (g)	Acidity (in terms of malic acid, g/L)	Brix	Concentration of Saccharose (g/L)	Concentration of Glucose (g/L)
Hu Bei Hai Tang	6	19	15.7	45	28
Yin Gyi Hai Tang	20	19	13.7	31	14
Xi Fu Hai Tang	5	15	14.9	29	22
Zhong Guo Ping Guo	143	22	11.0	9.7	23
Cui Guo	16	12	17.0	22	45
Fuji	256	3.0	11.7	25	27

[0034] As shown in Table 1, the ripe crab apples were smaller than the ripe apple fruit of cultivated species. Insofar as the general analysis values thus measured, none of them particularly characterized the crab apples.

[Example 2]

(Analysis of Polyphenol in Crab Apple Juice)

[0035] Various kinds of crab apple juices were prepared in the same manner as in the above Example 1. A total polyphenol content was measured by a Folin-Ciocalteu method and expressed also in terms of chlorogenic acid. Further, a total procyanidin content was measured by a Porter method and expressed also in terms of procyanidin B2. Other polyphenol components were measured quantitatively by high-performance liquid chromatography. The results are shown in Tables 2 and 3.

Table 2

Kind	Total Polyphenol Content (ppm)	Chlorogenic Acid (ppm)	Epicatechin (ppm)	Total Procyanidin Content (ppm)	PB1 (ppm)	PB2 (ppm)	PC1 (ppm)
Hu Bei Hai Tang	17442	104	517	13817	196	891	423
Yin Gyi Hai Tang	15678	731	538	11669	27	983	460
Xi Fu Hai Tang	9361	142	176	6420	45	272	125
Zhong Guo Ping Guo	3933	141	191	1147	51	109	39
Cui Guo	11583	629	345	7560	40	615	269
Adams Crab	23570	100	801	16827	81	1367	736
Geneva	6331	270	271	2085	86	351	128
Gorgeous	5671	64	21	1514	trace	80	14
Jay Darling	9417	325	484	6068	81	784	306
Eleyi	10466	566	558	9229	115	1023	365
Eley Purple Crab	14789	352	418	6374	45	845	379
Malus Robusta	12715	586	707	3901	203	669	326
Makamic Crab	13361	283	411	5379	169	731	294
Profusion	13792	208	442	7241	115	672	283
Royalty	26776	944	35	30847	trace	2296	323
Liset Crab	17334	22	511	21925	99	182	348
Red Splendor Crab	15934	152	20	20906	trace	trace	14
Tar Tan	5092	295	116	882	22	168	49
Lemoine Purple Crab	10156	136	342	3750	120	570	230
Fuji	2195	168	46	913	18	68	24

Table 3

Kind	Total Polyphenol Content (ppm)	Chlorogenic Acid (ppm)	Epicatechin (ppm)	Total Procyanidin Content (ppm)	PB1 (ppm)	PB2 (ppm)	PC1 (ppm)
Da Sha Guo	4753	427	113	1308	16	182	61
Hai Tang Hua	16017	193	557	10877	133	1020	424
Long Dong Hai Tang	6606	77	8	4798	2	13	5
Qin Guan	2124	217	3	559	2	2	trace
Sha Guo	4277	188	37	783	17	56	19
Xiang Hong Sha Guo	8159	498	189	4129	30	366	156
Xiao Huang Hai Tang	7383	188	92	4654	14	173	67
Yan Tai Sha Guo	6231	263	89	2963	20	152	48
Yin Gye Hai Tang	13243	569	321	7309	14	577	250
Yarlington Mill	7157	1039	452	1622	166	322	100
Aldenhams Purple Crab	9161	129	456	7267	162	619	271
Olga Crab	5104	176	144	3224	54	266	96

[0036] As shown in Table 2, the crab apples have a higher total polyphenol content than Fuji which is an apple to be eaten fresh. It is obvious that the Adams Crab and Royalty species contain about 10 times as much polyphenol as Fuji. Further, in terms of the components of the polyphenol, it is obvious that most of the crab apples contain chlorogenic acid and epicatechin in large quantity. In addition, it has been disclosed that most of the crab apples contain a large quantity of condensed tannins which are responsible for many functionalities of the apple fruit polyphenol. It has also been disclosed that of the condensed tannins, the contents of dimer fraction procyanidins B1 (PB1) and B2 (PB2) and a trimer fraction procyanidin C1 (PC1) which are known to have a hair-growing effect as disclosed in JP-A-8-503021 are also very high in most of the crab apples. Further, it is also obvious that by use of Royalty containing little procyanidin B1 (PB1), a purified product of the procyanidin B2 (PB2) can be obtained easily. As for Red Splendor Crab, the total procyanidin content is high, but neither PB1 nor PB2 is detected, and the content of PC1 is also low. Therefore, it can be said that this species is an excellent material for purifying procyanidin fractions (multimers larger than a trimer) other than PB1, PB2 and PC1.

[0037] Further, as shown in Table 3, Hai Tang Hua contains at least 10 times as much procyanidins as Fuji. The species also has high contents of PB1, PB2 and PC1. Yin Gye Hai Tang contains PB2 in much larger quantity than PB1. Since both PB1 and PB2 are a dimer, it is difficult to separate them, and yields thereof are low. However, by use of the species as a raw material, PB2 can be purified easily. On the other hand, Yarlinton Mill is excellent as a raw material from which PB1 can be separated and purified easily. Similarly, although Long Dong Hai Tang has a high total procyanidin content, it has low contents of PB1, PB2 and PC1. It can be said that this species is an excellent raw material to purify procyanidin fractions (multimers larger than a trimer) other than PB1, PB2 and PC1.

[Example 3]

(Analysis of Polyphenol in Juice of Crab Apple from Uyghur in China)

[0038] Apples produced in the Uyghur Province in China were collected, and polyphenol components in crab apple juices prepared by the same sample preparation process as used in the above Example 1 except that 600 ppm of sulfurous acid was added to Uyghur crab apples 1 and 2 and 350 ppm of sulfurous acid was added to an Uyghur crab apple 3 were measured. A total polyphenol content was determined by measuring absorbance at 280 nm and also expressed in terms of chlorogenic acid. Other components were quantified in the same manner as in the above Example 2. The results are shown in Table 4.



Table 4

Kind	Total Polyphenol Content (ppm)	Chlorogenic Acid (ppm)	Epicatechin (ppm)	Total Procyanidin Content (ppm)	PB1 (ppm)	PB2 (ppm)	PC1 (ppm)
Uyghur Crab Apple 1	16418	1591	1150	17376	203	1161	558
Uyghur Crab Apple 2	13209	1254	1130	16368	245	1222	454
Uyghur Crab Apple 3	11770	1232	1034	13691	197	1107	407

[0039] The Uyghur Crab Apples 1, 2 and 3 shown in Table 4 are crab apples produced in I-rei in the Uyghur Province. These apples are ones grown in an old growth forest of crab apples, and their species are not identified. All these crab apples contain a large quantity of polyphenol. Particularly, the contents of PB1 and PB2 which are a procyanidin dimer and the content of PC1 which is a trimer are very high. While a value obtained by dividing the total content of PB1, PB2 and PC1 by the total polyphenol content is 5% for Fuji, the value is 11.7% for the crab apple 1, 14.5% for the crab apple 2, and 14.5% for the crab apple 3. Since PB1, PB2 and PC1 have a hair-growing effect, it is understood that it is preferable to use these crab apples as raw materials so as to purify a fraction containing these 3 components.

[Example 4]

(Production of Polyphenol Fraction from Crab Apple Juice)

- 5 [0040] By use of a ripe crab apple produced in the Uyghur Province in China in 1999 as a raw material, a juice was prepared by the same sample preparation process as used in the above Example 1. 190 ml of the obtained juice was passed through a Sepabeads SP-850 resin column manufactured by Mitsubishi Chemical Corporation at room temperature, and the column was then washed with deionized water whose amount was three times as large as the volume of the column. After washing, a 50% ethanol aqueous solution whose amount was three times as large as the volume of the column was passed through the column so as to elute and recover a polyphenol from the resin. As a result of freeze-drying the obtained 50% ethanol fraction, 858 mg of polyphenol powder was obtained. As a result of quantifying polyphenol components in this powder by the same process as used in the above Example 2, it was found that in addition to 1.1% of catechin and 14.0% of epicatechin, 1.5% of procyanidin B1, 15.9% of procyanidin B2 and 6.8% of procyanidin C1 which are condensed tannins are contained. It was proved that a product containing a high concentration of polyphenol can be produced from an crab apple easily.

#### POSSIBILITY OF INDUSTRIAL UTILIZATION

- 20 [0041] As described above, according to the present invention, by use of a ripe crab apple as a raw material and a specific purification process, a fruit polyphenol rich in such components as condensed tannins (procyanidins), chlorogenic acid and epicatechin can be provided economically and efficiently.

#### Claims

- 25 1. A fruit polyphenol obtained by subjecting a crab apple fruit to pressing and/or extraction.
- 30 2. The fruit polyphenol of claim 1, wherein the crab apple is at least one selected from the group consisting of Hu Bei Hai Tang, Yin Gui Hai Tang, Xi Fu Hai Tang, Zhong Guo Ping Guo, Cui Guo, Ya Hai Tang, Ya Sha Guo, Ya Ping Guo, Adams Crab, Geneva, Gorgeous, Jay Darling, Eleyi, Eley Purple Crab, Malus Robusta, Makamic Crab, Profusion, Royalty, Liset Crab, Red Splendor Crab, Tar Tan, and Lemoine Purple Crab.
- 35 3. The fruit polyphenol of claim 1, wherein the crab apple is an apple produced in the Uyghur Province in China, Kazakhstan or Uzbekistan.
- 40 4. A process for producing a fruit polyphenol which comprises the steps of subjecting a crab apple fruit to pressing and/or extraction and then purifying the obtained juice and/or extract so as to obtain a polyphenol fraction.
- 45 5. The process of claim 4, wherein 350 to 3,000 ppm of sulfurous acid is added at the time of pressing the crab apple fruit.
- 50 6. The process of claim 4, wherein at least 100 ppm of sulfurous acid is added when a proper amount of alcohol is mixed into the crab apple fruit which is then crushed at the time of subjecting the crab apple fruit to extraction, and at least 50 ppm of sulfurous acid is added after preparation of a clear extract.
- 55 7. The process of claim 4, wherein the crab apple is at least one selected from the group consisting of Hu Bei Hai Tang, Yin Gui Hai Tang, Xi Fu Hai Tang, Zhong Guo Ping Guo, Cui Guo, Ya Hai Tang, Ya Sha Guo, Ya Ping Guo, Adams Crab, Geneva, Gorgeous, Jay Darling, Eleyi, Eley Purple Crab, Malus Robusta, Makamic Crab, Profusion, Royalty, Liset Crab, Red Splendor Crab, Tar Tan, and Lemoine Purple Crab.
8. The process of claim 4, wherein the crab apple is an apple produced in the Uyghur Province in China, Kazakhstan or Uzbekistan.

## INTERNATIONAL SEARCH REPORT

International application No.

PCT/JP01/07889

A. CLASSIFICATION OF SUBJECT MATTER Int.Cl. <sup>7</sup> A61K35/78, 31/353, A23L1/30, A61P17/14		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) Int.Cl. <sup>7</sup> A61K35/78, 31/353, A23L1/30, A61P17/14		
Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practicable, search terms used) STN (CA, BIOSIS, MEDLINE)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X Y	Vigorov, L. I., "Catechins in apples", Chemical Abstracts, July, 1969, Vol.71, No.3, page 69, 10263t (AN, 71:10263)	1,4 2,3
X Y	Dzhangaliev, A. D., "Vanity of forms crab apple trees of Kazakhstan and efficient use of them as chemiocotechnological indexes", Chemical Abstracts, February, 1974, Vol.80, No.7, page 208, 35957w (AN, 80:35957)	1,3,4 2,8
X Y	EP 657169 A1 (The Nikka Whisky Distilling Co., Ltd.), 14 June, 1995 (14.06.95), & AU 6899694 A1 & CA 2128293 A & JP 7-285876 A, (page 3, Par. No. [0014]) & CN 1121924 A & US 5994413 A	1,4,5 2,3,6-8
<input type="checkbox"/> Further documents are listed in the continuation of Box C. <input type="checkbox"/> See patent family annex.		
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Date of the actual completion of the international search 04 December, 2001 (04.12.01)		Date of mailing of the international search report 18 December, 2001 (18.12.01)
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